Degradation of chitosan for rice crops application

Norzita Yacob* Maznah Mahmud Norhashidah Talip Kamaruddin Hashim
Abdul Rahim Harun Khairul Zaman Hj Dahlan

Malaysian Nuclear Agency, 43000 Kajang, Selangor, MALAYSIA

Abstract A variety of techniques including chemical and enzymatic hydrolysis, and radiation degradation processes can be used to prepare low molecular weight chitosan. Degradation of chitosan by radiation can be carried out in solid state and liquid state. Radiation degraded polysaccharides has been reported to exhibit growth-stimulating activity like phytohormones thatinduce the promotion in germination, shoot and root elongation in variety of plants. In this study, the chitosan was irradiated in solid state (powder form) by gamma rays within the dose range of 25–75 kGy. And the irradiated chitosan was then irradiated in solution form in the presence of hydrogen peroxide. The effects of irradiation on the molecular weight and viscosity of the chitosan were investigated using Ubbelohde Capillary Viscometer. The molecular weight and viscosity of the chitosan decreased with increment of absorbed doses. In the presence of hydrogen peroxide, the molecular weight of chitosan could be further decreased. The effect of radiation degraded chitosan on the growth promotion of rice was investigated and it was shown during seedling period of 15 days for transplanting whereby the growth is 15%–20% faster than using chemicals growth promoters.

Key words Radiation, Degradation, Rice crops, Growth promoter

1 Introduction

Chitosan is a cationic polymer, which is the second most abundant polymer in nature following cellulose. Chitosan is a linear copolymer polysaccharide consisting of β(1-4)-linked 2-amino-2-deoxy-*D*-glucose (*D*-glucosamine) and 2-acetamido-2-deoxy-*D*-glucose (*N*-acetyl-*D*-glucosamine) units. Chitosan has been identified in its use in many primary industries such as: agriculture, paper, textiles, pharmaceutical, cosmetic and wastewater treatment[1–3]. It is an interesting biomaterial due to good biocompatibility, biodegradability, low toxicity, hemostatic potential, good film forming character and anti-infectional activity. The unique properties of chitosan arise from its amino groups that carry positive charges at pH values below 6.5, which enables its binding to negatively charged materials such as enzymes, cells, polysaccharides, nucleic acids, hair, and skin. However, in some fields (especially in medicine and food industry) the application of chitosan is limited by its high molecular weight and low solubility in aqueous media[4,5]. Low-molecular weight chitosan can be prepared by chemical[6], enzymatic[7] or radiation[8] degradation of high molecular weight chitosan.

Radiation has been found as one of the most popular tools for modification of polysaccharides. Without using any chemicals or high temperature treatment, radiation is known for its safer, environmental friendly and easier method to modify polymers. Natural polymers that have the potential to be modified such as cellulose, starch, guar gum, gum acacia, chitin-chitosan, alginates, carrageenans and their derivatives, which occur abundantly. These polymers are now being explored for potential applications in agriculture, food, medicine and cosmetic industries due to their unique structures, bio-degradability, biocompatibility and non-toxicity. Irradiation of natural polymer will lead to scission of main chain that can produce an oligomer which has lower molecular weight and viscosity. In some applications, for example in plant growth promoter

* Corresponding author. *E-mail address*: norzita@nuclearmalaysia.gov.my
Received date: 2012-12-31

and drug delivery system, low molecular weight of chitosan or alginate is needed to make the polymer working successfully.

The oligosaccharides derived from deploy-merization of polysaccharides through enzymatic hydrolysis have been reported to exhibit growth-stimulating activity like phyto hormones that induce the promotion in germination, shoot and root elongation in variety of plants[9–11]. Radiation degraded polysaccharides also show similar effects as plant growth promoter. For example, radiation degraded alginate solution (4%) at 100 kGy has been shown to have significantly enhanced growth for the rice seedlings in the hydrophonic systems[12]. The suitable range of concentration of degraded alginate between 20–100 ppm has impacted 15% and 60% weight gain of rice and peanuts, respectively. A similar study has been conducted by Relleve *et al.*[13] using irradiated carrageenan for rice seedlings under non-circulating hydrophonic condition.

The effect of radiation degraded poly-saccharide on the growth promotion of plant is not well understood. However, radiation degraded polysaccharide can be controlled to certain size of molecular weight to produce oligosaccharides that are mobile and easy for uptake by plant. In this paper, the effects of radiation processed oligochitosan on growth of rice seedlings and its uses as fungicide are reported.

2 Experimental

2.1 Materials

Chitosan powder was purchased from Nibong Tebal with the following properties, 90.6% of DDA, 10% water content and contain 0.1 ppm As, 0.1 ppm Cd, 0.8 ppm Cu, 3.16 ppm Ni and 0.1 ppm Pb. Hydrogen peroxide, H_2O_2, was purchased from Sigma, sodium acetate and acetic acid were purchased from Fisher Scientific while lactic acid was obtained from OFT Chemicals Sdn. Bhd. All reagents were used as received without any purification.

2.2 Preparation of oligochitosan

Chitosan powder was irradiated in PE plastic bags with gamma ^{60}Co source using Sinagama Facility (Nuclear Malaysia Agency) within the dose range of 25, 50 and 75 kGy and the dose rate of 2.15 $kGy{\cdot}h^{-1}$. The irradiated chitosan powder with optimum dose was then dissolved in lactic acid prior to irradiation using gamma rays from ^{60}Co at 12 kGy combined with a small amount of hydrogen peroxide. The irradiated chitosan powder later was dissolved in the lactic acid solution and kept overnight stirring for the complete hydration of chitosan.

2.3 Molecular weight and intrinsic viscosity measurement

Intrinsic viscosity and viscosity average molecular weight were examined using an automatic system Ubbelohde capillary type viscometer which allows the reading of flow times of the sample taken automatically without using stop watch. The capillary number is 531/10I and the measurement was conducted at (25±0.1)ºC. The reduced viscosity and inherent viscosity were plotted against chitosan concentration. The value of intrinsic viscosity can be calculated by extrapolating graph of reduced viscosity and inherent viscosity to zero concentration. The average of the two obtained intercept values was calculated. The intrinsic viscosity, η as function of viscosity average molecular weight, M_v is represented by Mark-Houwink-Sakurada equations.

$$\eta = KM_v^{\alpha} \quad (1)$$

where $K=3\times10^{-2}$ (mL/g) and $a=0.83$ determined in 0.2M CH_3COOH / 0.1 M CH_3COONa solution. The solution was prepared 24 h before doing the measurement and can only be used within 24 h.

2.4 Study of growth of rice seedling

Rice seeds of MR219 supplied by FECLRA Berhad were used through out of this study. Three different treatments of rice seeds were performed namely,

i. Rice seeds were soaked in commercial grade solution of growth promoter for 24 h as control (C).

ii. Rice seeds were soaked in oligochitosan (O) for 24 h.

iii. Rice seeds treated (i) were sprayed with commercial grade solution for nutrients.

iv. Rice seeds treated (ii) were sprayed with oligochitosan at day 8th and day 13th.

Rice seeds as control (C) and treated with Oligo (O), were planted in trays (~300 seeds per tray) containing rice husk ashas the planting media.

2.5 Field trail for blast study

For blast study, nine treatments were done on a total size of 24 hectares with 1.0 hectare size per plot and prepared in triplicates as follows:

3 Results and discussion

Radiation technology has been used to produce high performance polymeric materials with unique physical and chemical properties. Upon radiation, two main reactions which influence the final properties of polymer: (a) scission of main chain, also known as degradation and (b) cross-linking, the opposite process to degradation. The former will be followed by reduction in molecular weight while the later will cause an increment in molecular weight. Degradation process usually occurs when natural polymer is subjected to ionizing radiation. Study done by Ulanski and Rosiak[14] reported that chitosan irradiated in the solid state was only degraded and that crosslinking is negligible. Degradation rate of polysaccharide depends on many factors such as types of ionizing source for instance gamma rays, electron beam or X-rays, radiation dose and condition of the sample. Reduction of molecular weight was found to be lower when the sample was irradiated in liquid state[15].

3.1 Effect of irradiation on molecular weight

Figure 1 illustrates effect of radiation on chitosan in powder form after exposing to gamma radiation with various doses up to 75 kGy. The most pronounced decrease was at lower dose up to 50 kGy. At dose 50 kGy the molecular weight dropped from 67 352 Da to 14 946 Da. This reduction was due to chain scission of chitosan backbone, where degradation process took place. Further increment in irradiation dose did not compensate the decrement in molecular weight of chitosan.

Table 1 Treatment and description

Treatment	Description
T1	Control (without fungicide & without oligochitosan)
T2	Control (spray fungicide)
T3	Spray oligochitosan on leaves (20 ppm)
T4	Spray oligochitosan on leaves (40 ppm)
T5	Spray oligochitosan on leaves (100 ppm)
T6	Soak in oligochitosan (200 ppm) + Spray oligochitosan on leaves (20 ppm) on 20th, 30th and 40th days
T7	Soak in oligochitosan (200 ppm) + Spray oligochitosan on leaves (40 ppm) on 20th, 30th and 40th days
T8	Soak in oligochitosan (200 ppm) + Spray oligochitosan on leaves (100 ppm) on 20th, 30th and 40th days
T9	Spray oligochitosan on leaves (40 ppm) on 20th, 40th and 70th days

A drop of molecular weight was followed by decrement in intrinsic viscosity of chitosan. Changes of intrinsic viscosity of chitosan with irradiation dose were depicted in Fig.1.

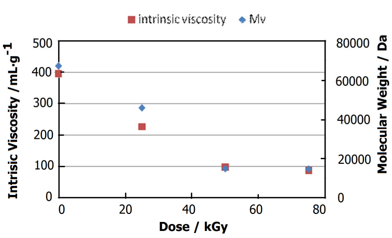

Fig.1 Changes of molecular weight and intrinsic viscosity as function of absorbed dose. Chitosan was irradiated in powder form using gamma radiation at 25, 50, and 75 kGy.

A sudden fall of intrinsic viscosity was observed at 50 kGy where the intrinsic viscosity reduced from 369.5 mL/g to 98.5 mL/g. After 50 kGy, intrinsic viscosity of chitosan was remained. These data could be suggested that the optimum radiation dose for chitosan powder was 50 kGy.

3.2 Effect of H_2O_2 on chitosan

A reduction of molecular weight can be further decreased by adding hydrogen peroxide, H_2O_2 as shown in Table 1. The irradiated powder chitosans were dissolved in lactic acid and then irradiated at 12 kGy in a presence of small amount of H_2O_2. It was

obvious from the result that irradiation in liquid state decreased the molecular weight rapidly to 8 034 Da as compared to the irradiated powder chitosan. A marked drop of molecular weight was observed for irradiated chitosan solution at dose 12 kGy in the presence of 0.1% H_2O_2 where the molecular weight reduced to 2 436 Da. With further increment in amount of H_2O_2, the molecular weight of chitosan decreased. It was obvious that with combination of gamma irradiation and hydrogen peroxide in liquid state, the decrement in molecular weight was higher compared to chitosan which irradiated using gamma irradiation alone. Under gamma irradiation in liquid state in the presence of H_2O_2, the primary reactions might occur as follows:

$$H_2O \rightarrow e_{aq}^-, OH\cdot, H\cdot, H_2O_2, H_2, H^+, OH^-, H_3O^+$$

$$H\cdot + H_2O_2 \rightarrow H_2O + \cdot OH$$

$$e_{aq}^- + H_2O_2 \rightarrow \cdot OH \rightarrow OH^-$$

$$H_2O_2 \rightarrow 2\cdot OH$$

$$R + \cdot OH \rightarrow \cdot R + H_2O$$

$$R + \cdot H \rightarrow \cdot R + H_2$$

$$\cdot R \rightarrow R_1 + R_2$$

OH radical is a much more powerful oxidant. This radical, which was derived from the radiolysis of water, and H_2O_2 will break the chitosan backbone and led to a shorter chains. Thus, the addition of hydrogen peroxide can further decrease the molecular weight of chitosan. The same phenomenon was found in the intrinsic viscosity observation when chitosan was irradiated in H_2O_2 solution.

Table 1 Effect of hydrogen peroxide on chitosan solution treated with gamma rays and gamma rays/H_2O_2. Chitosan powder was pre-irradiated in powder form at 50 kGy prior to irradiation in liquid format 12 kGy and 24 kGy

Sample	Mv/Da×10^3	Intrinsic viscosity, mL/g
12 kGy, without H_2O_2	8.034	53.5
24 kGy, witout H_2O_2	3.466	26.65
12 kGy, 0.1% H_2O_2	2.436	19.9
12 kGy, 0.3% H_2O_2	1.965	16.65

3.3 Rate of growth of rice seedling

Figure 2 shows that the rate of growth of rice seedling for rice seeds treated with oligochitosan is faster than the rice seeds treated with commercial grade growth promoter. The usual height of rice seedling for transplanting is around 14 cm at 15th day which is now can be achieved at 11th day by using oligochitosan. This will shorten the rice seedling period by 4 days prior to transplanting. During this period, no additional nutrients were added to the rice seeds which treated with oligochitosan. On the other hand, rice seeds treated with commercial grade growth promoter required 2 times spraying of nutrients.

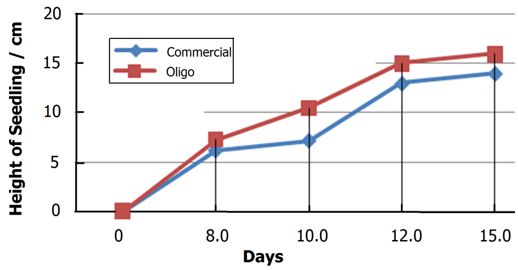

Fig.2 Rate of growth of rice seedling before transplanting.

3.4 Blast disease infection

From the blast lesion index against rice treatment as shown in Fig.3, it shows that the control (T1) has the highest rank of blast infection. Treatment with oligochitosan and fungicide showed lower average rank of blast infection as compared to control. T6, T7 and T8 with oligochitosan treatment on seed and leaf have given the lower number of plant infection with blast. The lowest rank of blast infection is at treatment 7 (Soak in oligochitosan (200 ppm)+Spray oligo-chitosan on leaves (40 ppm) on 20th, 30th and 40th days). This study has shown a significant effect of oligochitosan on rice plant from having a severe blast infection in the field.

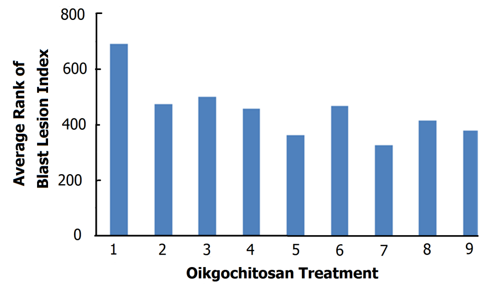

Fig.3 Comparison of average rank among nine oligochitosan treatments

4 Conclusion

Radiation technology has been proven as a method that can be used to reduce the molecular weight of chitosan and other natural polymer. The molecular weight of chitosan has been reduced with increment of

radiation dose followed by reduction in intrinsic viscosity. The radiation treatment on chitosan in the presence of hydrogen peroxide could enhance the reduction its molecular weight effectively. Oligo-chitosan was shown to act as growth promoter during rice seedling period of 1st day to 15th day. From blast lesion index, oligochitosan treatment has shown an effective elicitor which can protect rice plant from blast disease.

References

1 Muzzarelli, R A A. Carbohydr Polym, 1996, **29:** 309–316.

2 Berger J, Reist M, Mayer J M, *et al.* Eur J Pharm Biopharm, 2004, **57:** 35–52.

3 Kurita K, Sanna T, Iwakura Y. J Appl Polym Sci, 1979, **23:** 511–515.

4 Minagawa T, Okamura Y, Shigemasa Y, *et al.* Carbohydr Polym, 2007, **67:** 640–644.

5 Biskup R C, Rokita B, Ulanski P, *et al.* Nucl Instrum Meth Phys Res B, 2005, **236:** 383–390.

6 Hasegawa M, Isogai A, Onabe F. Carbohydr Polym, 1993, **20:** 279–283.

7 Ilyina A V, Tikhonov V E, Albulov A I, *et al.* Process Biochem, 2000, **5:** 563–568.

8 Won-Seok C, Kil-Jin A, Dong-Wook L, *et al.* Polym Degrad Stab, 2002, **78:** 533–538.

9 Akiyama H, Endo T, Nakakita R, *et al.* Biotech Biochem, 1992, **56:** 335–356.

10 Natsume M, Kamo Y, Hirayama M, *et al.* Carbohydr Res, 1994, **256:** 187–197.

11 Tomoda Y, Umemura K, Adachi T. Biosci Biotech Biochem, 1994, **58:** 203–208.

12 Nguyen Q, Nagasawa N, Le X, *et al.* Radiat Phys Chem, 2000, **59:** 97–101.

13 Relleve L S, Abad L V, Aranilla C T, *et al.* Biological activities of radiation degraded carrageenan. Proceedings of a symposium on Radiation Technology in Emerging Industrial Applications, Beijing, China, Nov 6-10, IAEA-SM-365/24, 2000, 98–108.

14 Ulanski P, Rosiak. Phys Chem, 1992, **39:** 53–57.

15 Fumio Y. Radiation degradation and crosslinking of polysaccharides and its application, Radiation processing of polysaccharides, IAEA-TECDOC-1422, November 2004, Vienna, Austria.